

## CLAIMS

What is claimed is:

✓ A method of analyzing the sequence of a polynucleotide of interest, comprising the steps of:

- annealing a polynucleotide of interest to free oligonucleotide primers having known sequences of N nucleotides in length to generate annealed primers;
- subjecting the annealed primers to a single base extension reaction to extend the annealed primers by the addition of a terminating nucleotide;
- observing the identity of each terminating nucleotide that has been added to the annealed primers.

✓ 2. A method of analyzing the sequence of a polynucleotide of interest, comprising the steps of:

- annealing a polynucleotide of interest to oligonucleotide primers having known sequences of N nucleotides in length under hybridization conditions, to generate annealed primers;
- subjecting the annealed primers to a single base extension reaction which comprises providing to the annealed primers nucleotides corresponding to each of the four bases, to extend the annealed primers by the addition of a terminating nucleotide;
- observing the identity and location of each terminating nucleotide that has been added to the annealed primers.

✓ 3. A method of analyzing the sequence of a polynucleotide of interest, comprising the steps of:

- attaching an array of oligonucleotide primers having known sequences of N nucleotides in length to a solid support at known locations;
- annealing the polynucleotide of interest to the array of oligonucleotide primers to generate annealed primers;
- subjecting the annealed primers to a single base extension reaction to extend the annealed primers by the addition of a terminating nucleotide;

d) observing the identity and location of each terminating nucleotide within the array on the solid support.

4. A method of analyzing the sequence of a polynucleotide of interest, comprising the steps of:

- a) attaching an array of oligonucleotide primers having known sequences of N nucleotides in length to a solid support at known locations;
- b) annealing the polynucleotide of interest to the array of oligonucleotide primers to generate annealed primers;
- c) subjecting the annealed primers to a single base extension reaction to extend the annealed primers by the addition of a terminating nucleotide;
- d) selecting a starting annealed primer;
- e) observing the identity and location of the terminating nucleotide which has been added to the starting annealed primer, to determine the next nucleotide in sequence;
- f) selecting a second annealed primer which has the same nucleotide sequence as nucleotides 2 through N of the starting annealed primer nucleotide plus the next nucleotide in sequence as determined in step (e), and
- g) repeating steps (e) and (f), using the second annealed primer as the starting annealed primer for each repetition, to determine the sequence of the polynucleotide of interest.

5. A method of analyzing the sequence of a polynucleotide of interest, comprising the steps of:

- a) attaching an array of oligonucleotide primers, having known sequences of N nucleotides in length to a solid support at defined locations;
- b) annealing the polynucleotide of interest to the array of oligonucleotide primers under hybridization conditions, to generate annealed primers;
- c) subjecting the annealed primers to a single base extension reaction which comprises providing to the annealed primers nucleotides corresponding to each of the four bases, to extend the annealed primers by the addition of a terminating nucleotide;
- d) observing the identity and location of each terminating nucleotide within the array on the solid support.

✓ 6. A method of analyzing the sequence of a polynucleotide of interest, comprising the steps of:

- attaching an array of oligonucleotide primers, having known sequences of N nucleotides in length to a solid support at defined locations;
- annealing the polynucleotide of interest to the array of oligonucleotide primers under hybridization conditions, to generate annealed primers;
- subjecting the annealed primers to a single base extension reaction which comprises providing to the annealed primers nucleotides corresponding to each of the four bases, to extend the annealed primers by the addition of a terminating nucleotide;
- selecting a starting annealed primer;
- observing the identity and location of the terminating nucleotide which has been added to the starting annealed primer, to determine the next nucleotide in sequence;
- selecting a second annealed primer which has the same nucleotide sequence as nucleotides 2 through N of the starting annealed primer nucleotide plus the next nucleotide in sequence as determined in step (e), and
- repeating steps (e) and (f), using the second annealed primer as the starting annealed primer for each repetition, to determine the sequence of the polynucleotide of interest.

7. The method of any one of Claims 1 to 6, wherein the single base extension reaction comprises subjecting the annealed primers to a reaction mixture comprising a polymerase and nucleotides corresponding to each of the four bases.

8. The method of any one of Claims 5 to 7, wherein the nucleotides corresponding to each of the four bases are mutually distinguishable.

9. The method of Claim 8, wherein three of the four nucleotides are differently labelled.

10. The method of Claim 9, wherein the three differently labelled nucleotides are fluorescently labelled.

11. The method of any one of Claims 1 to 10, further comprising analyzing the sequence of the complementary polynucleotide of interest.

12. The method of any one of Claims 1 to 11, wherein the terminating nucleotides are dideoxynucleotides.

13. The method of any one of Claims 1 to 12, wherein the length N of the oligonucleotide primers is between 7 and 30 inclusive.

14. The method of any one of Claims 1 to 13, wherein the length N of the oligonucleotide primers is between 20 and 24 inclusive.

15. The method of any one of Claims 1, 2, 13 or 14, wherein the oligonucleotide primers comprise oligonucleotide primers of different lengths.

16. The method of any one of Claims 1 to 15, wherein observing the identity and location of a terminating nucleotide comprises the use of a charge coupled device or a photomultiplier tube.

17. The method of any one of Claims 3 to 14 or 16, wherein the terminating nucleotides are removed from the annealed primers after completed analysis to prepare the solid support for reuse.

18. The method of any one of Claims 1 to 17, wherein the terminating nucleotides are dinucleotides.

19. An apparatus for analyzing the sequence of a polynucleotide of interest, comprising a solid support having attached thereto at defined locations an array of oligonucleotide primers having known sequences.

20. The apparatus of Claim 19, wherein the oligonucleotide primers are attached to the solid support by a specific binding pair.

21. The apparatus of Claim 20, wherein the specific binding pair is biotin and a molecule selected from the group consisting of: avidin and streptavidin.

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